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Effects of clenbuterol and propranolol on muscle mass

Evidence that clenbuterol stimulates muscle β-adrenoceptors to induce hypertrophy

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1. A single subcutaneous injection of clenbuterol hydrochloride (0.125 mg/kg body wt.) to female Wistar rats produced a rapid increase in muscle cyclic AMP and lactate concentrations and a decrease in muscle glycogen concentrations. These changes are characteristic of muscle β-adrenoceptor stimulation and were abolished by intraperitoneal injection of propranolol (12.5 mg/kg) 15 min before clenbuterol administration. 2. When this dose of clenbuterol was injected twice daily, the changes in muscle metabolite concentrations which followed its acute administration persisted until day 7 of treatment, and were accompanied by increases in muscle mass, body weight and muscle protein synthesis rate (k_s). When the clenbuterol injections were preceded by propanolol injections (12.5 mg/kg administered according to the protocol described above), or if animals were treated with propranolol only, the values of these variables were not significantly different from those of sham-injected controls. 3. In rats fed on a semi-synthetic diet (PW3) supplemented with 2 mg of clenbuterol/kg of diet for 7 days, the muscle mass was greater than that of rats fed on unsupplemented PW3. The increased muscle mass was accompanied by increased muscle lactate and decreased muscle glycogen concentrations. When PW3 was supplemented with 2 mg of clenbuterol/kg and 200 mg of propranolol/kg, the increase in muscle mass remained, but decreased muscle glycogen concentrations and increased muscle lactate concentrations were also observed. 4. These data are consistent with the hypothesis that clenbuterol influences muscle growth via β-adrenoceptor stimulation.

INTRODUCTION

Numerous studies have demonstrated that administration of β-adrenergic agonists to a variety of species causes increased muscle growth and alterations in body composition (Yang & McElligott, 1989). Particular interest has focused on the effects of the β_2-agonist clenbuterol [4-amino-(t-butylamino)methyl-3,5-dichlorobenzyl alcohol]. In rats fed on a normal diet (Reeds *et al.*, 1986) or one which is protein-deficient (Rothwell & Stock, 1987), this agent causes an increase in muscle protein deposition and a decrease in the ratio of total body protein to total body fat. Clenbuterol also reverses denervation-induced muscle atrophy (Maltin *et al.*, 1987*b*; Zeman *et al.*, 1987).

A key question regarding the biochemical basis of these effects concerns whether clenbuterol and similar drugs alter body composition by β-adrenoceptor stimulation or by another mechanism. There is evidence to support both possibilities; for example, Zeman *et al.* (1988) reported that chronic treatment with clenbuterol caused hypertrophy of fast-twitch muscle fibres, whereas the β_2-antagonist butoximine decreased fast-twitch fibre size, suggesting that clenbuterol influences muscle growth by β-adrenoceptor stimulation. In contrast, Maltin *et al.* (1987*a*) found that addition of propranolol to clenbuterol-supplemented diets did not attenuate the hypertrophy of innervated (Maltin *et al.*, 1987*a*) or denervated (Maltin *et al.*, 1989) soleus muscles induced by clenbuterol. Furthermore, the dose of propranolol employed in those studies inhibited the effects of clenbuterol on energy expenditure and on fat and liver mass (Reeds *et al.*, 1988). These reports suggest that clenbuterol may exert a physiological response despite β-blockade; however, no results were presented to demonstrate that, in those experiments, propranolol fully blocked the β-adrenergic effects of clenbuterol on muscle.

In this study our aim was to resolve the question of whether or not it is necessary for β-adrenoceptor stimulation to occur for clenbuterol to exert its effects on body composition. We have therefore re-investigated the effects of treatment with clenbuterol and propranolol on muscle growth. To ensure that propranolol fully blocked the β-adrenoceptor-stimulatory activity of clenbuterol in our experiments, we have measured indices of the degree of muscle β-adrenoceptor stimulation during different treatments. We have also examined the effects of clenbuterol and propranolol treatment on muscle protein synthesis rate (k_s).

EXPERIMENTAL

Materials

L-Phenyl[2,3-^{3}H]alanine and the cyclic AMP assay kit (TRK. 432) were purchased from Amersham International, Amersham, Bucks., U.K. Clenbuterol hydrochloride was generously given by Beecham Pharmaceuticals, Epsom, Surrey, U.K. All other chemicals and biochemicals were purchased from Sigma Chemical Co., Poole, Dorset, U.K., or B.D.H., Poole, Dorset, U.K. We are grateful to Dr. C. A. Maltin of the Rowett Research Institute, Aberdeen, for supplying PW3 diet. Female Wistar rats were maintained on a 12 h-light/12 h-dark cycle, with water available *ad libitum*.

Abbreviations used: k_s, protein synthesis rate; k_d, protein degradation rate.

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Metabolite determinations

Neutralized $HClO_4$ extracts of muscle and liver (Lowry & Passonneau, 1972) were prepared for determination of lactate concentrations (Gutman & Wahlefeld, 1974). Glycogen concentrations in muscle and liver were measured by the method of Keppler & Decker (1974). Protein was assayed by the method of Lowry *et al.* (1951). Muscle and liver samples were extracted for assay of tissue cyclic AMP concentrations by the procedure described by Albano *et al.* (1974). Briefly, frozen tissue was homogenized in 10 vol. of 0.5 M-$HClO_4$ containing 25% (v/v) ethanol. The homogenate was centrifuged at 1200 *g* for 20 min (4 °C) and a sample of the neutralized supernatant was evaporated to dryness and then resuspended in the assay buffer (50 mM-Tris/4 mM-EDTA, pH 7.5). Recoveries of cyclic AMP were 95–107% with this procedure. The cyclic AMP assay was performed with an Amersham kit.

Determination of muscle protein synthesis rate

This was done by the method of Garlick *et al.* (1980), with the modifications described by Jepson *et al.* (1986). Briefly, conscious rats were injected intraperitoneally with a solution of 150 mM-[^{3}H]phenylalanine (35 μCi/ml) in 150 mM-NaCl (2 ml/100 g body wt.). Some 15 min later the animals were killed by a blow to the head. Both hind-limbs were then rapidly skinned and placed in a mixture of ice and water. The exact time (t) from injection of radioisotope to immersion of limbs was noted. The gastrocnemius and plantaris group of muscles was removed and placed in liquid N_2 before determination of the specific radioactivity of free (S_A) and protein-bound (S_B) phenylalanine. Muscle k_s was calculated from the equation

$$k_s(\%/\text{day}) = \frac{S_B}{0.9S_A \cdot t} \times 100\,\%$$

(Jepson *et al.* 1986). The muscle RNA concentration was determined by the method of Munro & Fleck (1969).

Animal manipulations

Injection protocol. In Expts. 1, 2 and 3 animals were injected intraperitoneally (1 ml/100 g body wt.) either with 150 mM-NaCl (vehicle) or with an equivalent volume of vehicle containing propranolol hydrochloride (12.5 mg of propranolol hydrochloride/kg body wt.). At 15 min after the intraperitoneal injection, each animal was subcutaneously injected (0.2 ml/100 g body wt.) either with vehicle or with an equivalent volume of vehicle containing clenbuterol (0.125 mg of clenbuterol hydrochloride/kg body wt.). Solutions of clenbuterol and propranolol were prepared immediately before injection.

Expt. 1. Female Wistar rats (104–131 g) were treated once only according to the protocol described above, i.e. they were injected with either vehicle or clenbuterol only or with both propranolol and clenbuterol. Samples of gastrocnemius and soleus muscles and of liver were removed from groups of three or four anaesthetized (sodium pentobarbital, 6 mg/kg) animals 0.5, 1, 2 and 5 h after the subcutaneous injection. Tissue samples were rapidly frozen in liquid N_2 before analysis of cyclic AMP, lactate and glycogen concentrations.

Expt. 2. For this, 20 rats weighing 118 ± 8 g (S.D.) were divided into four groups of equal mean body weight, consisting of five animals per group. Standard laboratory diet was available to animals *ad libitum* throughout the experimental period. Food intakes and body weights were monitored daily. Twice daily (first pair of injections 09:00–10:00 h, second pair of injections 16:30–17:30 h) for 6 days, all animals were subjected to subcutaneous and intraperitoneal injections of clenbuterol and/or propranolol or their respective vehicles. One group was treated with vehicle only, a second group with clenbuterol only (2 × 0.125 mg/kg per day), a third group with propranolol only (2 × 12.5 mg/kg per day) and a fourth group with both propranolol and clenbuterol. Final intraperitoneal and subcutaneous injections were administered between 09:00 and 10:00 h 7 days after the treatment commenced. At 20 min after the final subcutaneous injection, the animals were anaesthetized (as described above). Then 10 min later gastrocnemius and soleus muscles (from the left hind-limb) and a sample of liver were rapidly excised and frozen in liquid N_2 before metabolite analyses. The gastrocnemius and plantaris group of muscles and the soleus muscle from the contralateral limb were carefully dissected and weighed, and then frozen in liquid N_2 for subsequent determination of protein content.

Expt. 3. For this, 16 female Wistar rats (123 ± 6 g) were divided into four groups of equal mean body weight, consisting of four animals per group. The procedure for injecting the different animal groups was identical with that in Expt. 2. At 30 min after the final subcutaneous injection on the morning of day 7 of treatment, muscle protein synthesis rates and RNA concentrations were determined.

Expt. 4. For this, 20 female Wistar rats (104–113 g) were fed on the semi-synthetic powdered diet PW3 (Pullar & Webster, 1977) *ad libitum* for 4 days. Each rat was then allocated to one of four groups of equal mean body weight (126 g) consisting of five rats per group. One group was maintained on PW3 diet, a second group PW3 containing 2 mg of clenbuterol/kg of diet, a third group PW3 containing 200 mg of propranolol/kg of diet, and a fourth group PW3 containing clenbuterol and propranolol at the doses stated above. After consuming the diets for 7 days, the animals were anaesthetized, and muscles and liver were removed as described in the protocol for Expt. 2.

RESULTS AND DISCUSSION

Acute effects of clenbuterol and propranolol injection (Expt. 1)

Within 0.5 h after a single subcutaneous injection of clenbuterol there was an increase of some 2-fold in the cyclic AMP concentration of gastrocnemius muscle, which persisted for at least 5 h (Fig. 1). The cyclic AMP concentration of liver was unaltered by injection of clenbuterol (results not shown). Increased muscle cyclic AMP concentrations in response to β-adrenoceptor stimulation have previously been observed (see, e.g., Posner *et al.*, 1965; Bowman *et al.*, 1985). Over the 5 h period there was also a progressive depletion of gastrocnemius and soleus muscle glycogen concentrations (Fig. 1*b*) and an elevation of muscle lactate concentrations (Fig. 1*c*), which achieved statistical significance 2 h after clenbuterol injection. The muscle and liver cyclic AMP,

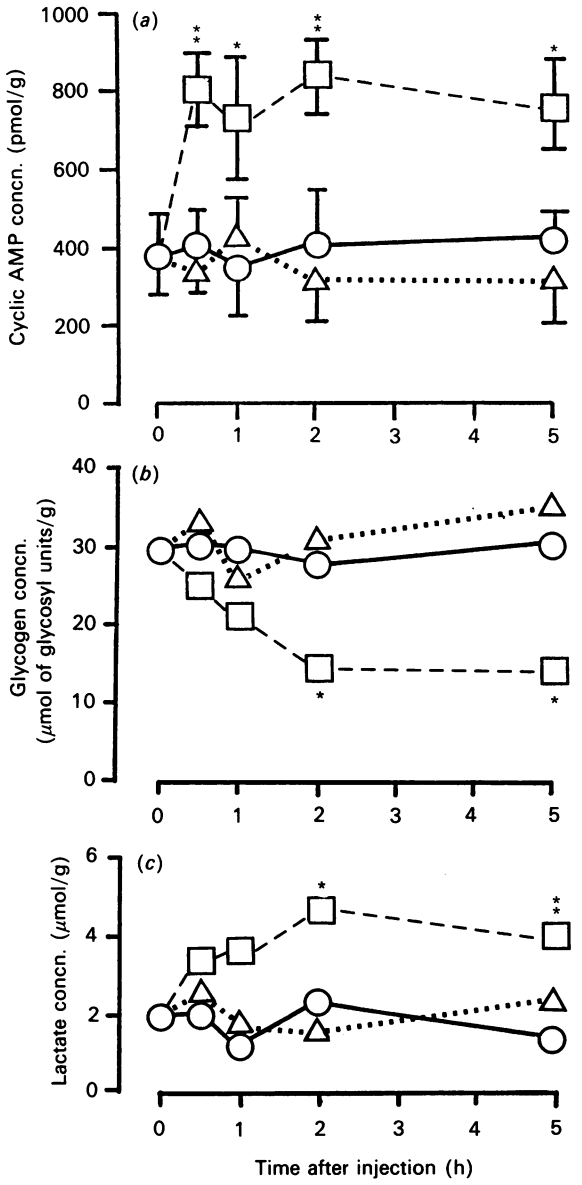

Fig. 1. Acute effects of clenbuterol and propranolol administration on gastrocnemius-muscle metabolite concentrations (Expt. 1)

Propranolol (12.5 mg/kg body wt.) (△) or an equivalent volume of vehicle (150 mM-NaCl) (○, □) was administered intraperitoneally to groups of three or four female Wistar rats. At 15 min after the intraperitoneal injection, either clenbuterol (0.125 mg/kg body wt.) (△, □) or an equivalent volume of vehicle (○) was administered subcutaneously. Gastrocnemius-muscle samples were obtained from anaesthetized animals at intervals thereafter. The cyclic AMP (*a*), glycogen (*b*) and lactate (*c*) concentrations of the muscle samples were determined. The results in (*a*) are means ± S.D.; error bars have been omitted from (*b*) and (*c*) for reasons of clarity: *($P < 0.05$) and **($P < 0.001$) indicate statistically significant differences from the group injected with vehicle only.

lactate and glycogen concentrations of injected controls were almost identical with those measured in non-injected anaesthetized animals (results not shown).

The effects of clenbuterol on muscle cyclic AMP, lactate and glycogen concentrations were totally abolished by propranolol administration (Fig. 1). These experiments therefore establish that, when the protocol used in the present study was employed, at least some of the consequences of clenbuterol-induced muscle β-adrenoceptor stimulation were abolished by propranolol.

The effects of clenbuterol and propranolol on muscle lactate and glycogen concentrations observed in the present study *in vivo* are in accord with previous work, performed *in vitro*, on the effects of muscle β-adrenoceptor stimulation. Challis *et al.* (1984) found that addition of isoprenaline to incubated rat soleus muscles treated with insulin produced an increase in glycolytic rate and a decrease in the rate of glycogen synthesis. Both effects of isoprenaline were propranolol-sensitive. Richter *et al.* (1982) showed that adrenaline stimulated glycogenolysis and lactate production in perfused muscle in the resting state and after electrical stimulation.

It is probable that clenbuterol, isoprenaline and adrenaline stimulated muscle β-adrenocepters, resulting in increased adenylate cyclase activity with a consequent elevation of cyclic AMP concentration (Helmreich *et al.*, 1976). Increased cyclic AMP would, in turn, activate cyclic-AMP-dependent protein kinase, resulting in alterations to the phosphorylation state of glycogen synthase and phosphorylase such that accelerated glycogenolysis and decreased glycogen synthesis ensued (Cohen, 1978).

Effects of clenbuterol and propranolol injection for 7 days on body composition (Expt. 2)

Twice-daily injections of clenbuterol for 7 days caused an increase in body-weight gain and an increase in the weight of gastrocnemius plus plantaris muscles and of soleus muscles (Table 1). The ratio of muscle weight to body weight was also increased in both muscle types by clenbuterol treatment. None of these variables was significantly different from those of the injected controls in animals treated with propranolol alone. In animals treated with clenbuterol and propranolol, the changes in body weight and body composition observed in animals treated with clenbuterol alone were abolished. This finding contrasts with that by Maltin *et al.* (1987*a*), who reported that the effects of dietary clenbuterol administration on muscle growth were unaltered by addition of propranolol to a clenbuterol-supplemented diet. The heart weight and heart-weight/body-weight ratio were unaltered by clenbuterol or propranolol; thus, if clenbuterol exerted any effects on the heart in the present study, they did not result in gross cardiac hypertrophy. Food intakes and muscle protein concentrations (Table 1) were essentially unaltered by treatment with the drugs.

Effects of clenbuterol and propranolol injection for 7 days on muscle and liver cyclic AMP, lactate and glycogen concentrations (Expt. 2)

In animals which had been treated with clenbuterol for 7 days there was, as observed after acute clenbuterol treatment, a substantial elevation of gastrocnemius-muscle cyclic AMP concentrations (Table 2). Liver cyclic AMP concentrations remained unaltered by clenbuterol treatment. It is unclear why both acute and chronic clenbuterol administration should have produced rises in muscle, but not liver, cyclic AMP concentrations, since both tissues possess β_2-adrenoceptors (Levitski, 1981). It is possible that, in rat liver, either β_2-adrenoceptors are present at a concentration which is insufficiently great to

Table 1. Effects of clenbuterol and propranolol injection for 7 days on body composition (Expt. 2)

Results are given as means ± S.D. for five rats. Animals were injected intraperitoneally with propranolol or vehicle and subcutaneously with clenbuterol or vehicle. Injections were given twice daily for 6 days, with muscles, liver and heart being sampled 30 min after a final injection given on day 7 of treatment. Further details are given in the text. *($P < 0.05$) and **($P < 0.001$) indicate statistically significant differences from animals injected with vehicle only.

Muscles	Measurement	Injected controls	Clenbuterol only	Propranolol only	Clenbuterol + propranolol
	Body wt. gain (g)	18 ± 5	36 ± 3**	23 ± 4	25 ± 4
	Chow intake (g/day)	17 ± 4	16 ± 3	18 ± 3	17 ± 2
Gastrocnemius and plantaris	Wet wt. (mg)	874 ± 96	1069 ± 53*	809 ± 86	880 ± 61
	Wet wt./body wt. (mg/g)	6.08 ± 0.34	6.69 ± 0.25*	5.55 ± 0.48	5.93 ± 0.35
	Protein concn. (mg/g wet wt.)	189 ± 7	193 ± 10	182 ± 9	186 ± 11
Soleus	Wet wt. (mg)	66 ± 6	78 ± 4*	64 ± 6	67 ± 5
	Wet wt./body wt. (mg/g)	0.46 ± 0.01	0.49 ± 0.02*	0.44 ± 0.02	0.45 ± 0.02
	Protein concn. (mg/g wet wt. of muscle)	193 ± 11	203 ± 16	191 ± 18	192 ± 21
Heart	Wet wt. (mg)	568 ± 42	605 ± 45	572 ± 49	576 ± 56
	Wet wt./body wt. (mg/g)	3.72 ± 0.36	3.77 ± 0.22	3.92 ± 0.29	3.88 ± 0.37

Table 2. Effects of clenbuterol and propranolol injection for 7 days on muscle and liver metabolite concentrations (Expt. 2)

Results are given as means ± S.D. for five rats. Treatment of the animals was described in the legend to Table 1. Analyses were performed on extracts of frozen tissue. Further details are given in the text. *($P < 0.05$) and **($P < 0.001$) indicate statistically significant differences from animals treated with vehicle only.

Muscle	Measurement	Injected controls	Clenbuterol only	Propranolol only	Clenbuterol + propranolol
Gastrocnemius	Cyclic AMP (pmol/g)	422 ± 120	835 ± 75**	222 ± 59	225 ± 139
	Glycogen (μmol of glycosyl units/g)	30.6 ± 4.9	17.3 ± 5.4**	37.2 ± 4.5	30.5 ± 4.4
	Lactate (μmol/g)	2.1 ± 0.7	4.9 ± 1.2*	2.3 ± 0.4	2.0 ± 1.0
Soleus	Glycogen (μmol of glycosyl units/g)	21.7 ± 3.4	14.1 ± 2.9**	23.8 ± 4.1	24.7 ± 6.9
	Lactate (μmol/g)	1.6 ± 0.4	4.8 ± 0.9*	2.0 ± 0.7	1.8 ± 0.4
Liver	Cyclic AMP (pmol/g)	504 ± 126	605 ± 107	421 ± 113	489 ± 196
	Glycogen (μmol of glycosyl units/g)	245 ± 44	322 ± 41*	196 ± 37	266 ± 53
	Lactate (μmol/g)	2.1 ± 0.6	2.1 ± 0.7	2.0 ± 0.2	1.3 ± 1.0

produce a detectable rise in cyclic AMP concentration, or they are of a subclass which is not stimulated by clenbuterol.

Chronic administration of the β_2 agonist also resulted in decreased gastrocnemius- and soleus-muscle glycogen concentrations, with liver glycogen concentrations being elevated. It is probable that the latter finding reflects increased Cori cycling (Cori, 1931), a suggestion which is supported by the increased muscle lactate concentrations observed after clenbuterol treatment.

In animals treated for 7 days with clenbuterol and propranolol or with propranolol alone, cyclic AMP, glycogen and lactate concentrations in muscle and liver did not differ significantly from those observed in injected controls. It may thus be inferred that, in this experiment, propranolol blocked the stimulation of muscle β-adrenoceptors by clenbuterol in addition to blocking the effect of clenbuterol to induce muscle hypertrophy.

Effects of clenbuterol and propranolol injection for 7 days on gastrocnemius-muscle protein synthesis rate (Expt. 3)

Chronic clenbuterol treatment also resulted in an increased k_s in gastrocnemius muscles (Table 3). The RNA concentration of gastrocnemius muscles (i.e. the 'protein synthetic capacity') from clenbuterol-treated animals was increased, as was the amount of protein synthesized per unit of RNA per day (k_{RNA}) (Millward, 1980). These data suggest that both the number of ribosomes per muscle and the rate of peptide synthesis per ribosome were increased by the drug. The effects of clenbuterol and propranolol on body weight in this experiment were similar to those in Expt. 2, and food intakes were similar in all experimental groups.

The finding that clenbuterol increased muscle k_s is in accord with that by Emery *et al.* (1984), but differs from

Fig. 3. Effects of clenbuterol and propranolol injection for 7 days on gastrocnemius-muscle protein-synthetic rates and RNA concentrations (Expt. 3)

Results are given as means ± S.D. for four rats. Treatment of the animals was as described in the legend to Table 1. Protein synthesis rates were measured 30 min after final subcutaneous injections. Further details are given in the text. *($P < 0.05$) indicates a statistically significant difference from animals injected with vehicle only.

	Injected controls	Clenbuterol only	Propranolol only	Clenbuterol + propranolol
k_s (%/day)	14.65 ± 1.67	20.97 ± 3.23*	12.65 ± 2.93	13.11 ± 1.98
RNA concn. (μg/g of protein)	9.6 ± 1.4	12.2 ± 1.0*	8.5 ± 1.7	10.03 ± 1.0
k_{RNA} (g of protein/day per g of RNA)	15.20 ± 0.9	17.2 ± 1.1*	14.9 ± 1.3	13.1 ± 1.8

Table 4. Effects of dietary administration of clenbuterol and propranolol for 7 days of body composition (Expt. 4)

Results are given as means ± S.D. for five rats. Animals were fed on semi-synthetic diet PW3 for 4 days and were then either maintained on PW3 or offered PW3 supplemented with clenbuterol only, propranolol only or clenbuterol and propranolol. Tissues were sampled from anaesthetized rats on day 7 after being offered PW3 or a supplemented diet. Further details are given in the text. *($P < 0.05$) and **($P < 0.001$) indicate statistically significant differences from animals maintained on PW3 only.

Muscle	Measurement	PW3	PW3 + clenbuterol	PW3 + propranolol	PW3 + clenbuterol + propranolol
	Body wt. gain (g)	29 ± 3	38 ± 7	31 ± 4	34 ± 3
Gastrocnemius and plantaris	Wet wt. (mg)	918 ± 20	1182 ± 167*	913 ± 77	1119 ± 125*
	Wet wt./body wt. (mg/g)	5.77 ± 0.11	6.66 ± 0.24**	5.41 ± 0.23	6.45 ± 0.23*
	Protein concn. (μg/g wet wt.)	187 ± 11	193 ± 9	186 ± 12	190 ± 14
Soleus	Wet wt. (mg)	72 ± 3	86 ± 10*	72 ± 5	86 ± 9*
	Wet wt./body wt. (mg/g)	0.45 ± 0.01	0.48 ± 0.02*	0.43 ± 0.02	0.50 ± 0.02*
	Protein concn. (mg/g wet wt.)	197 ± 10	189 ± 9	200 ± 16	194 ± 9
Heart	Wet wt. (mg)	619 ± 95	713 ± 69	658 ± 87	654 ± 80
	Wet wt./body wt. (mg/g)	4.11 ± 0.31	4.04 ± 0.22	3.92 ± 0.29	3.77 ± 0.33

the findings by Reeds *et al.* (1986), who concluded that dietary clenbuterol administration did not alter muscle k_s. The measurements by Emery *et al.* (1984) were performed 1 h after subcutaneous injection of 1 mg of clenbuterol/kg body wt.; thus the experimental protocol was similar to that of the present study. In contrast, Reeds *et al.* (1986) measured protein synthesis rate after clenbuterol intakes of some 200 μg/kg body wt. per day, with the drug being administered by dietary supplementation. It seems likely that the dose and/or the route of clenbuterol administration may influence the effect of the drug on muscle protein synthesis rate, although it is not possible to offer an explanation for this interesting finding.

The increased muscle RNA/protein ratio observed in the present study after clenbuterol treatment has not previously been reported in intact animals. In the previous study in which administration of clenbuterol caused increased muscle k_s (Emery *et al.*, 1984), RNA/protein ratios were not reported. When clenbuterol was administered in such a way as to cause muscle hypertrophy but no change in muscle k_s, the RNA/protein ratio was unaltered (e.g. Reeds *et al.*, 1988). However, the increased muscle protein content and k_s of clenbuterol-treated denervated soleus muscles (Maltin *et al.*, 1987*b*) was accompanied by a transient increase in muscle RNA/protein ratio. Thus it appears that under circumstances in which clenbuterol administration causes increased muscle k_s the muscle RNA/protein ratio is also increased.

In the present study, the increased muscle protein deposition observed in animals treated with clenbuterol may have been partly the consequence of an altered rate of muscle protein degradation (k_d). No method exists whereby the muscle k_d may be directly determined in the rat *in vivo*, and our data do not provide an adequate basis from which to make an indirect estimate of the rate of this process. Indeed, to arrive at a quantitative determination of k_d in muscle, it would theoretically have been necessary to measure the rate of protein synthesis at all times throughout the entire experimental period.

Effects of dietary supplementation with low doses of clenbuterol and propranolol (Expt. 4)

Maltin *et al.* (1987*a*, 1989) have reported that the clenbuterol-induced muscle growth of innervated and denervated soleus muscles, respectively, was propranolol-insensitive. In an attempt to resolve the apparent conflict between this result and the data reported above, we repeated the experiments of Maltin *et al.* (1987*a*). Our findings regarding the effects of dietary clenbuterol

Table 5. Effects of dietary administration of clenbuterol and propranolol for 7 days on muscle and liver metabolite concentrations (Expt. 4)

Results are given as means ± S.D. for five rats. Treatment of the animals was as described in the legend to Table 4. Analyses were performed on extracts of frozen tissue. Further details are given in the text. *($P < 0.05$) and **($P < 0.001$) indicate statistically significant differences from animals maintained on PW3 only.

Muscle	Measurement	PW3	PW3 + clenbuterol	PW3 + propranolol	PW3 + clenbuterol + propranolol
Gastrocnemius	Cyclic AMP (pmol/g)	386 ± 61	541 ± 99	352 ± 40	566 ± 107
	Glycogen (μmol of glycosyl units/g)	35.6 ± 6.9	16.5 ± 5.7**	36.9 ± 4.3	16.3 ± 3.8**
	Lactate (μmol/g)	1.9 ± 0.8	4.7 ± 1.0*	2.3 ± 0.4	5.2 ± 0.8*
Soleus	Glycogen (μmol of glycosyl units/g)	24.9 ± 3.6	13.7 ± 2.4**	26.8 ± 3.0	12.4 ± 3.1**
	Lactate (μmol/g)	2.4 ± 0.7	5.1 ± 0.9**	2.0 ± 0.8	4.7 ± 0.6*
Liver	Cyclic AMP (pmol/g)	402 ± 70	511 ± 142	387 ± 66	417 ± 61
	Glycogen (μmol of glycosyl units/g)	263 ± 53	307 ± 31	204 ± 46	324 ± 27
	Lactate (μmol/g)	2.8 ± 0.6	2.0 ± 0.9	1.7 ± 1.0	2.4 ± 0.5

(2 mg/kg of diet) and propranolol (200 mg/kg of diet) administration on soleus muscles are in agreement with these of the previous investigators, i.e. clenbuterol induced soleus-muscle hypertrophy, which was unaltered by inclusion of propranolol in the clenbuterol-supplemented diet (Table 4). Furthermore, we found that clenbuterol induced propranolol-insensitive hypertrophy in the gastrocnemius and plantaris muscle groups. Unlike Maltin *et al.* (1987*a*), we found muscle protein concentration to be unaltered by clenbuterol treatment.

No significant changes in gastrocnemius (or liver) cyclic AMP concentrations relative to those of the control group were observed (Table 5), although the mean cyclic AMP concentration in gastrocnemius muscles from animals treated with clenbuterol only or with clenbuterol plus propranolol was increased. However, in both gastrocnemius and soleus muscles of animals which had been treated with clenbuterol only, or with clenbuterol plus propranolol, glycogen concentrations were significantly decreased and lactate concentrations were significantly elevated. These data suggest the possibility that the dose of propranolol used in this last study was insufficiently great to block the β-adrenoceptor-stimulatory activity of clenbuterol completely. Although significant elevation of the muscle cyclic AMP concentration was not apparent on day 7 of treatment with the β_2-agonist, the depletion of muscle glycogen and elevation of muscle lactate concentrations strongly suggest the possibility that a degree of β-adrenoceptor stimulation had taken place. It is possible that, at the time of muscle sampling, a localized increase of muscle cyclic AMP was present, which was great enough to stimulate cyclic AMP-dependent protein kinase, but was of insufficient magnitude to be detectable at the level of the whole muscle. Alternatively, there might have been an increase in muscle cyclic AMP concentration during administration of clenbuterol, and of clenbuterol plus propranolol, which did not persist until day 7 of treatment.

General discussion

In an experimental system in which effects of clenbuterol which are characteristic of β-adrenergic stimulation of muscle were abolished by propranolol, the effects of clenbuterol on both muscle growth and protein synthesis rate were also abolished by the β-blocking agent (Expts. 1–3 of the present study). However, when propranolol was administered in such a way that some of the characteristic changes induced by muscle β-adrenoceptor stimulation remained, propranolol did not alter the effects of clenbuterol on muscle growth (Expt. 4 of the present study). The data in the present paper therefore support the suggestion that the effects of clenbuterol on muscle protein deposition are a consequence of β-adrenoceptor stimulation by the drug.

Our study is the first of which we are aware in which the effects of clenbuterol on muscle cyclic AMP concentrations and carbohydrate metabolism have been evaluated. A notable feature of the present study was that the effects of clenbuterol on muscle cyclic AMP, lactate and glycogen concentrations were detectable after twice-daily injection of the drug for 7 days. This finding suggests that the β-adrenergic effects of clenbuterol on muscle may follow a different time course from the effects of the drug on other tissues, since Herbert *et al.* (1985) demonstrated that gastric infusion of clenbuterol produced an effect on sheep heart rate for only 36 h, whereas the effects of the drug on nitrogen retention persisted.

The β-adrenergic effects of clenbuterol on muscle may also be less sensitive to propranolol than the β-adrenergic effects of the drug on other tissues. Reeds *et al.* (1988) reported that the dose of propranolol employed in Expt. 4 of the present study inhibited the effects of clenbuterol on cardiac, fat and liver mass and on energy expenditure, whereas the effects of clenbuterol on muscle growth remained. However, Expt. 4 of the present study has demonstrated that under these experimental conditions the effects of clenbuterol on muscle glycogen and lactate concentrations (characteristic β-adrenergic effects) persisted despite propranolol administration.

The Wistar rats used in the present study may show different sensitivities to the muscle-growth and β-effects of clenbuterol compared with the Hooded Lister animals used by Maltin *et al.* (1987*a*, 1989) and Reeds *et al.* (1988). However, in Expt. 4 of our study, clenbuterol-induced cardiac hypertrophy was not statistically significant, whereas in the study of Reeds *et al.* (1988) a significant degree of hypertrophy was observed. Because the effects of clenbuterol on heart mass are thought to be

β-mediated (Reeds *et al.*, 1988) Hooded Lister rats appear to be, if anything, more sensitive to the β-effects of clenbuterol than the animals used in our experiments. If this is indeed the case, then Expt. 4 of our study would have been more likely to achieve β-blockade than the study of Maltin *et al.* (1987*a*), since equal doses of propranolol were administered in both experiments.

The evidence presented above suggests that the muscle hypertrophy induced by clenbuterol is β-mediated, but provides no clue as to the mechanism through which β-stimulation and increased muscle growth are causally linked. Clearly further studies are required to delineate the mechanism through which β-adrenergic agonists exert their effects on muscle growth and body composition.

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